

# CORE CURRICULUM IN NEPHROLOGY

## Renal Function Testing

Mitchell H. Rosner, MD, and W. Kline Bolton, MD

---

### ASSESSMENT OF GLOMERULAR FILTRATION RATE

---

#### Why Test Renal Function?

- Patients with kidney disease have few signs and symptoms early in disease course; laboratory evaluation may be only way of detecting disease
- Tests should detect abnormalities early enough to allow corrective therapy
- Important for measuring renal disease progression and efficacy of therapies
- Help predict when renal replacement therapy may be necessary
- Aid in appropriate dosing of medications
- Tests that best detect abnormalities of renal function measure glomerular filtration rate (GFR)

#### Measurement of GFR

- GFR cannot be measured directly
- Remarkably constant in single individual under constant conditions
- Large variation between individuals with large spread of normal values
- Causes of interpatient variability include:
  - Body size: GFR conventionally factored by 1.73 m<sup>2</sup>
  - Sex: GFR approximately 8% higher in males
  - Race

---

*From the Department of Internal Medicine, Division of Nephrology, University of Virginia Health System, Charlottesville, VA.*

*Received July 6, 2005; accepted in revised form August 16, 2005.*

*Originally published online as doi:10.1053/j.ajkd.2005.08.038 on December 5, 2005.*

*Address reprint requests to Mitchell H. Rosner, MD, Division of Nephrology, Box 800133, University of Virginia Health System, Charlottesville, VA 22908. E-mail: mhr9r@virginia.edu*

*© 2005 by the National Kidney Foundation, Inc.*

*0272-6386/05/4701-0022\$30.00/0*

*doi:10.1053/j.ajkd.2005.08.038*

- Age: age-related decline in GFR, 0.75 to 1.0 mL/min/1.73 m<sup>2</sup> (0.01 to 0.02 mL/s/1.73 m<sup>2</sup>) per year
- Pregnancy: GFR elevated as much as 50% in first trimester and onward; returns toward normal by 4 to 8 weeks postpartum
- Protein intake: GFR higher in patients on high-protein diet
- Diurnal variation: values tend to be about 10% higher in afternoon than at night
- Antihypertensive therapy: secondary to lowering of blood pressure; variable effect not directly predictable
- States associated with hyperfiltration: diabetes, obesity, acromegaly
- Clearance (C): the rate at which an indicator substance is removed from plasma per unit concentration; specifies a volume from which all of a substance is removed per unit time
- For a substance Z cleared by renal elimination:

$$C_z = U_z \times V / P_z$$

Where U<sub>z</sub> is urinary concentration of z, P<sub>z</sub> is plasma concentration of Z, and V is urine flow rate

- If substance z is freely filtered and only excreted by GFR, then:  $GFR = U_z \times V / P_z$
- Thus, plasma concentration of indicator is inversely related to GFR and GFR can be assessed from plasma concentration
- Requires an ideal filtration marker (Table 1) to ensure that elimination of substance is completely dependent on GFR

#### Use of Exogenous Markers to Measure GFR

- Techniques:
  - Urine collection with time-averaged plasma concentration of marker substance, or
  - Plasma clearance determined by either a continuous infusion or bolus method
- Marker substances:
  - Inulin (molecular weight [MW], 5,000 d):

**Table 1. Characteristics of an Ideal Marker for GFR Measurement**

---

Constant rate of production (or for exogenous marker can be delivered intravenously at a constant rate)  
 Freely filterable at the glomerulus (minimal protein binding)  
 No tubular reabsorption  
 No tubular secretion  
 No extrarenal elimination or metabolism  
 Availability of an accurate and reliable assay  
 For exogenous marker: safe, convenient, readily available, inexpensive, and does not influence GFR (physiologically inert)

---

- Fructose polymer, freely filtered, neither reabsorbed nor secreted by tubules, physiologically inert
- Determinations are labor intensive, limiting its widespread use
- Radionuclide-labeled markers:
  - Clearance is determined as amount of indicator injected divided by integrated area of plasma concentration curve over time (as determined by formulas)
  - Most commonly used: <sup>125</sup>I-iothalamate and <sup>51</sup>Cr-ethylenediaminetetra-acetic acid (EDTA) (plasma levels) or <sup>99m</sup>Tc-mercaptoacetyl triglycine (MAG<sub>3</sub>) (gamma counter)
- Radiocontrast markers:
  - Iothalamate sodium, iohexol (safest), diatrizoate meglumine; measure iodine levels

**Use of Endogenous Markers to Determine GFR**

- Creatinine (MW, 113 d)
- Metabolism:
  - Generated in muscle by nonenzymatic conversion of creatine and phosphocreatinine
  - Generation is proportional to muscle mass and is relatively constant
  - Important role of liver in formation of creatinine through methylation of guanidine aminoacetic acid
- Levels vary according to diurnal and menstrual variations, race, and diet (and method of meat preparation)
- Excretion rate (urinary creatinine excretion × V) mg/kg/d:
  - Males:  $28.2 - 0.172 \times \text{age}$

- Females:  $21.9 - 0.115 \times \text{age}$
- Serum levels can be affected by numerous factors (Table 2)
- Not an ideal marker since it also is excreted by tubular secretion:
  - Proportion of total creatinine clearance (C<sub>cr</sub>) due to tubular secretion increases as GFR decreases and C<sub>cr</sub> leads to GFR overestimation by approximately 10 mL/min/1.73 m<sup>2</sup> (0.17 mL/s/1.73 m<sup>2</sup>)
  - In some subjects (eg, those with sickle cell anemia), this GFR overestimation can be much greater
- Because of reciprocal relationship between GFR and serum creatinine (SCr), a large change in GFR is initially required to raise SCr levels from normal; however, once SCr is elevated, small changes in GFR will raise it exponentially more
- 1/SCr better reflects magnitude of GFR decline
- Laboratory determination of creatinine:
  - Jaffé reaction: creatinine reacts with picrate under alkaline conditions to form a chromogen:
    - Positive interference from glucose, ascorbic acid, uric acid, acetoacetate, pyruvate, ketoacids; results in creatinine value 20% higher than true value
    - Negative interference; high serum bilirubin levels may cause spuriously low SCr values
  - Enzymatic kinetic alkaline picrate method; less interference from noncreatinine chromogens and much more accurate
  - Enzymatic assays (amidohydrolase method) have similar precision to the kinetic method

**Table 2. Factors Affecting SCr**

Increase SCr	Decrease SCr
Ketotic states, hyperglycemia (Jaffé)*	Dietary protein restriction
Cephalosporins (Jaffé)*	Muscle wasting, malnutrition
Flucytosine (enzymatic method)*	Bilirubin (Jaffé)*
Cimetidine, trimethoprim (block secretion)	Renal disease
Vigorous exercise	Advanced age
Ingesting cooked meats	Female sex
	Advanced liver disease

\*These factors affect the measurement process directly.

- All methods lose precision in lower range of assay; thus, SCr is insensitive in determining small changes of GFR from normal
- Autoanalyzers often are calibrated to different creatinine standards with substantial variation (up to 0.4 mg/dL [35  $\mu$ mol/L]) between creatinine values from different laboratories
- Creatinine clearance:
  - Calculated from 24-hour urine sample and single SCr (assumes steady state)
  - Inaccuracies result from incomplete urine collections
  - Not valid for patients not in steady state
  - Overestimates GFR due to tubular secretion of creatinine (especially important at lower GFRs)
  - Cimetidine blocks tubular secretion of creatinine and thus increases accuracy of  $C_{cr}$ ; optimal dosing is not clear and blockade of tubular secretion often incomplete, thus limiting this technique
- SCr as a marker of GFR:
  - Elevated level usually indicates reduced GFR
  - Normal level does not exclude possibility of reduced GFR:
    - Factors may keep creatinine generation rate low (Table 2)
    - SCr may thus remain within normal ranges in these patients despite a significant GFR reduction
    - Especially important in patients with chronic kidney disease where patients restrict protein intake and may blunt rise in SCr despite falls in GFR
    - Liver disease where SCr may be low despite poor GFR
- Formulae for estimating GFR using SCr:
  - Equations include factors related to creatinine generation and excretion (age, sex, race, body size)
  - Rely on patients being in steady state
  - Major limitation is due to variation in measurement of SCr with kinetic Jaffé reaction and autoanalyzers and from intrinsic variability of SCr
  - Cockcroft-Gault equation predicts  $C_{cr}$  as  $C_{cr} = (140 - age) \times (weight)/(72 \times SCr)$  (multiply by 0.85 if female):
    - Overestimates  $C_{cr}$  in vegetarians and patients who are malnourished, on low-protein diet, obese, or edematous
    - In patients with renal disease, predicted  $C_{cr}$  overestimates GFR at low values
- Modification of Diet in Renal Disease (MDRD) equation predicts GFR more accurately than measured  $C_{cr}$  and is preferred method:  $GFR = 170 \times [SCr (mg/dL)]^{-0.999} \times [age]^{-0.176} \times [0.762 \text{ if patient is female}] \times [1.18 \text{ if patient is black}]$ :
  - Not validated across diverse ethnic populations, in patients age >60 years or <18 years, with diabetes
  - May underestimate GFR in stage 1 chronic kidney disease and overestimate GFR in stages 4 and 5
  - Failure to calibrate creatinine assay to laboratory that developed the estimating equation can introduce systematic error in estimated GFR, particularly at a high GFR
- Among children, the Schwartz and Counahan-Barratt formulae provide clinically useful estimates of GFR
- Note: Use of SCr to estimate GFR relies on steady state; in certain circumstances, clearance methods may be more reliable:
  - Extremes of age and body size
  - Severe malnutrition or obesity
  - Diseases of skeletal muscle
  - Paraplegia or quadriplegia
  - Vegetarian diet
  - Rapidly changing SCr
- Urea (MW, 60 d):
  - One of first indicators used to measure GFR
  - Shares few features of ideal marker and is poor measure of GFR
  - Freely filtered but reabsorbed in proximal and distal nephron (urea clearance is less than GFR); urea reabsorption is substantial in states of decreased renal perfusion
  - Urea production is variable and largely dependent on protein intake
  - Many variables affect urea level (Table 3)
  - State of diuresis affects urea clearance more than  $C_{cr}$  and is useful in differential diagnosis of acute renal failure (ARF)

**Table 3. Factors Affecting Serum Urea Nitrogen**

Increase Serum Urea	Decrease Serum Urea
Dehydration	Volume expansion
Reduced renal perfusion (heart failure)	Pregnancy
Increased dietary protein	SIADH
Catabolic states:	Restriction of dietary protein
Fever	Liver disease
Trauma	Advanced renal disease
GI bleeding	
Tetracyclines	
Corticosteroids	

Abbreviations: GI, gastrointestinal; SIADH, syndrome of inappropriate secretion of antidiuretic hormone.

where blood urea nitrogen–creatinine ratio is increased when causes are prerenal

- Cystatin C (MW, 13,000 d):
  - Cysteine proteinase inhibitor produced by all nucleated cells at constant rate
  - Freely filtered and then absorbed and catabolized by renal tubules
  - No significant urinary excretion
  - Measurement by particle-enhanced nephelometric immunoassay (PENIA) with high degree of precision and accuracy
  - Normal adult values range from 0.54 to 1.55 mg/dL
  - Most but not all studies show that serum cystatin C is better index of GFR than SCr alone
  - Factors that affect serum level of cystatin and that are independent of GFR are still controversial and possibly include older age, male sex, smoking, higher weight, thyroid disease, and higher levels of C-reactive protein
  - Additional studies, across diverse populations to determine value as index of renal function, still required

#### ADDITIONAL READING

1. Bauer JH, Brooks CS, Burch RN: Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. *Am J Kidney Dis* 2:337-346, 1982
2. Levey AS: Nephrology forum: Measurement of renal function in chronic renal disease. *Kidney Int* 38:167-184, 1990
3. Nilsson-Ehle P, Grubb A: New markers for the determination of GFR: Iohexol clearance and cystatin C serum concentration. *Kidney Int Suppl* 46:S17-S19, 1994
4. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular

filtration rate from serum creatinine: A new prediction equation. *Ann Intern Med* 130:461-470, 1999

5. Grubb AO: Cystatin C—Properties and use as diagnostic marker. *Adv Clin Chem* 35:63-99, 2000
6. Knight EL, Verhave JC, Spiegelman D, et al: Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 65:1416-1421, 2004
7. Coresh J, Astor BC, McQuillan G, et al: Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 39:920-929, 2002
8. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31-34, 1976
9. Luke RG: Urea and the BUN. *N Engl J Med* 305:1213-1215, 1981
10. Herrington D, Drusano G, Smalls U, et al: False elevation in serum creatinine levels. *JAMA* 252:2962, 1984 (letter)

11. Ibrahim H, Mondress M, Tello A, et al: An alternative formula to the Cockcroft-Gault and the Modification of Diet in Renal Diseases formulas in predicting GFR in individuals with type 1 diabetes. *J Am Soc Nephrol* 16:1051-1060, 2005

12. Froissart M, Rossert J, Jacquot C, et al: Predictive performance of the Modification of Diet in Renal Disease and Cockcroft-Gault equations for estimating renal function. *J Am Soc Nephrol* 16:763-773, 2005

#### ASSESSMENT OF RENAL PLASMA FLOW (RPF)

- Infrequently needed in routine clinical practice
- Derived from rate of clearance of a marker that is totally extracted from plasma after first pass through kidney; this yields RPF
- Renal blood flow (RBF) can be obtained by dividing RPF by (1 - hematocrit)
- $\rho$ -aminohippurate (PAH) is most widely used marker
- PAH clearance gives the effective RPF (ERPF) because part the RBF perfuses a region that does not contribute to PAH excretion; PAH clearance is about 10% lower than actual RPF
- Mean values of ERPF are 650 mL/min/1.73 m<sup>2</sup> in males and 600 mL/min/1.73 m<sup>2</sup> in females
- Other markers that can be used include determination of plasma clearance of a radioactive marker such as <sup>131</sup>I-hippuran or MAG<sub>3</sub>

#### ADDITIONAL READING

1. Cole BR, Giangiacoimo J, Ingelfinger JR, Robson AM: Measurement of renal function without urine collection: A critical evaluation of the constant-infusion technic for determi-

nation of inulin and para-aminohippurate. *N Engl J Med* 287:1109-1114, 1972

2. Smith HW, Goldring W, Chasis H: The measurement of the tubular excretory mass, effective blood flow and filtration rate in the normal human kidney. *J Clin Invest* 17:263-278, 1938

---

## ASSESSMENT OF TUBULAR FUNCTION

---

### Concentration and Dilution of Urine: Methods

- Relies on measurement of urine and plasma osmolalities:
  - Osmolality is related to number of particles in solution and is independent of charge, size, or density
  - Methods for determination of osmolality include:
    - Freezing point depression: extent to which freezing point of a solution is depressed below that of distilled water is linearly related to osmolality:
      - Advantages: precision and not influenced by excretion of protein or iodinated contrast agents
      - Disadvantages: expense and labor required
    - Urine-specific gravity:
      - Measures not only total number of particles but also relative size and density of particles and thus is not a measure of urine osmolality
      - Refrigeration of urine or excretion of large amounts of protein, glucose, or contrast agents will result in specific gravity increases
      - Measured with hydrometer or refractometer
    - In general, urine osmolality of 50 mOsm/kg (mmol/kg) is approximately equivalent to a specific gravity of 1.000; 300 mOsm/kg, a specific gravity of 1.010; 800 mOsm/kg, a specific gravity of 1.020
    - Dipstick measurement of urine specific gravity:
      - Assumes that as amount of solutes in urine increases, there will be a concomitant increase in amount of ions (salts)
      - As concentration of ions increases, a polyacid impregnated on the strip

releases H<sup>+</sup> ions and an acid-base indicator changes color

- Imprecise and should not be used in any formal testing

### Assessment of Renal Concentrating Ability

- Necessary for patients with polyuria or hyperosmolality
- Assessed with water deprivation test, which assess integrated response of pituitary and kidney
- Normal water deprivation for 18 to 24 hours leads to urine osmolality >900 mOsm/kg in most healthy persons
- Failure to concentrate urine is further assessed with exogenous vasopressin
- Central diabetes insipidus: urine osmolality increases in response to vasopressin
- Nephrogenic diabetes insipidus: no change in urine osmolality in response to vasopressin

### Assessment of Renal Diluting Capacity

- Useful in patients with hypoosmolality
- Can be assessed with water loading, but test has low diagnostic yield and is seldom useful in clinical practice
- Defect in diluting process is usually evident when serum hypoosmolality coexists with a urine that is not maximally dilute and does not require formal testing

### ADDITIONAL READING

1. Sweeney TE, Beuchat CA: Limitations of methods of osmometry: Measuring the osmolality of biological fluids. *Am J Physiol* 264:R469-R480, 1993

2. Zerbe RL, Robertson GL: A comparison of plasma vasopressin measurements with a standard indirect test in the differential diagnosis of polyuria. *N Engl J Med* 305:1539-1546, 1981

### Assessment of Urinary Acidification

- Useful in diagnosis of non-gap metabolic acidosis: determining extrarenal versus renal causes (renal tubular acidosis) and the specific renal cause
- pH:
  - Usually measured with reagent test strip with normal range from 4.5 to 7.8
  - Should be measured quickly after sample is obtained

- Most accurate when obtained using H<sup>+</sup>-specific electrode on urine collected under oil
- Alkaline pH: urea-splitting organisms, vegetarian diet, diuretics, nasogastric suction, vomiting, alkali therapy
- Acidic pH: metabolic acidosis, high protein diet
- Urine pH itself has little diagnostic information
- Urine anion gap ( $[\text{Na}^+ + \text{K}^+] - \text{Cl}^-$ ) estimates urine  $\text{NH}_4^+$  concentration and renal response to acidosis:
  - Positive urine anion gap: decreased urine ammonium production (renal tubular acidosis), presence of unmeasured ketoacids, hippurate, benzoate, or penicillin-derivative antibiotics (piperacillin, ticarcillin)
  - Negative urine anion gap: increased urine ammonium production and extrarenal source of acidosis
- Urine osmolal gap:
  - Useful when urine anion gap is positive and unclear whether increased excretion of unmeasured anion is responsible
  - Calculation requires measurement of urine osmolality and the urine sodium, potassium, urea, glucose
  - Gap = measured – calculated urine osmolality:
    - Calculated urinary osmolality =  $2 \times (\text{Na} + \text{K}) + (\text{urea}/2.8) + (\text{glucose}/18)$
    - Positive osmolal gap consistent with increased ammonium production
- Alkali loading test: test urine pH and serum bicarbonate response to bicarbonate infusion (proximal renal tubular acidosis):
  - Infusion of sodium bicarbonate at 0.5 to 1.0 mEq/kg/h
  - Urine pH, even if initially acidic, will increase rapidly once resorptive threshold for bicarbonate is exceeded; urine pH will be  $>7.5$  and fractional excretion of bicarbonate  $>15\%$  to  $20\%$  as plasma bicarbonate approaches normal
- Urine-blood  $\text{PCO}_2$ :
  - Examined while urine is alkaline

- In healthy subjects, alkalization of urine is associated with urine  $\text{PCO}_2$  approximately 30 mm Hg greater than blood
- Urine values similar to blood  $\text{PCO}_2$  are consistent with classical distal renal tubular acidosis
- Other tests of distal acidification:
  - Sodium sulfate infusion
  - Response to loop diuretic

#### ADDITIONAL READING

1. Halperin ML, Richardson RM, Bear R, et al: Urine ammonium: The key to the diagnosis of distal renal tubular acidosis. *Nephron* 50:1-4, 1980
2. Batlle DC: Segmental characterization of defects in collecting tubule acidification. *Kidney Int* 30:546-554, 1986
3. Sabatini S, Kurtzman NA: Pathophysiology of the renal tubular acidoses. *Semin Nephrol* 11:202-211, 1991

#### Assessment of Tubular Function in ARF

- Useful in differential diagnosis of ARF, especially in distinguishing prerenal from renal causes
- Tubular function impaired with acute tubular necrosis
- Fractional excretion (FE) of sodium, urea, and uric acid allow assessment of tubular function (Table 4):
  - Fractional excretion of substance x =  $[(\text{U}/\text{plasma concentration of substance x})/(\text{U}/\text{Scr})] \times 100$
  - Caveats include:
    - Low FE sodium may be seen early in course of tubular injury accompanying rhabdomyolysis, sepsis, administration of radiocontrast materials, nonoliguric forms of ARF, nonsteroidal anti-inflammatory drug use, acute interstitial nephritis, and with acute glomerulonephritis; also may be seen with acute tubular necrosis in setting of vasoconstrictive states such as congestive heart failure and cirrhosis
    - High FE sodium may be seen in prerenal states in which there is impaired renal tubular reabsorption of sodium such as with diuretic use, bicarbonaturia, glucosuria, recent intravenous contrast administration, salt-wasting ne-

phropathy, and mineralocorticoid deficiency

- Biomarkers of renal tubular injury are available:
  - Their current role is more experimental than diagnostic
  - No current “gold standard”
  - Sample biomarkers include brush border enzymes such as *N*-acetyl- $\beta$ -glucosaminidase, adenosine deaminase-binding protein, as well as proteins such as kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and sodium-hydrogen exchanger 3 (NHE3)
- Measurement of low-molecular-weight proteins that are readily filtered and usually reabsorbed by proximal tubule:
  - May appear in urine when there is tubular injury
  - These proteins include  $\beta_2$ -microglobulin, amylase, lysozyme, and retinol-binding protein
  - These measurements are not routinely useful

#### ADDITIONAL READING

1. Carvounis CP, Nisar S, Guro-Razuman S: Significance of the fractional excretion of urea in the differential diagnosis of acute renal failure. *Kidney Int* 62:2223-2229, 2002
2. Rabb H: Evaluation of urinary markers in acute renal failure. *Curr Opin Nephrol Hypertens* 7:681-686, 1998
3. Miller TR, Anderson RI, Linas SL, et al: Urinary diagnostic indices in acute renal failure: A prospective study. *Ann Intern Med* 88:47-57, 1978

**Table 4. Urinary Indices in ARF**

Urinary Study	Prerenal	Renal
Specific gravity	>1.020	~1.010
Urinary osmolality (mOsm/kg)	>500	<300
Urine sodium (mEq/L)	<20	>20
Fractional sodium excretion (%)	<1	>2
Fractional uric acid excretion (%)	<7	>15
Fractional urea excretion	<35	>35
Low-molecular-weight proteins ( $\beta_2$ -microglobulin, lysozyme)	Low	High
Brush-border enzymes ( <i>N</i> -acetyl- $\beta$ -glucosaminidase)	Low	High

NOTE. To convert osmolality in mOsm/kg to mmol/kg, multiply by 1; sodium in mEq/L to mmol/L, multiply by 1.

## ASSESSMENT OF PROTEINURIA

### General Guidelines

- Spot urine specimens can be used to detect and monitor proteinuria
- First morning specimens are preferred method
- Screening for proteinuria can be accomplished with either standard urine dipsticks (total proteinuria) or albumin-specific dipsticks
- Patients with positive dipstick tests should have confirmation with quantitative measurement (albumin-creatinine ratio, protein-creatinine ratio, or 24-hour collection)
- Monitoring proteinuria in patients with chronic kidney disease should be performed with quantitative measurements
- Total protein-creatinine ratio is acceptable method if albumin-creatinine ratio is high (>500 mg to 1,000 mg/g)

### Measurement of Total Urine Protein:

#### Methodology

- Semiquantitative tests for total urine protein:
  - Precipitation tests use 5% sulfosalicylic acid, concentrated nitric acid, or 10% trichloroacetic acid to precipitate proteins in urine; quantity of precipitate is graded from 0 to +4
  - Dipstick test uses a pH indicator dye (tetrabromophenol blue) buffered to pH of 3.0; proteins act to change color of indicator dye with color varying depending on concentration of protein present:
    - Detect total protein >10 to 20 mg/dL
  - Tests are insensitive but have high specificity
  - Dipstick false-negative results with immunoglobulin light chains, tubular proteins that have positive charges (Table 5)
  - False-positive results (Table 5)
  - They detect an abnormal concentration, not an abnormal excretion rate
  - Useful for screening purposes
- Quantitative tests for total urine protein:
  - 24-hour collection:
    - Urinary protein is precipitated with trichloroacetic or sulfosalicylic acid



**Table 5. Causes of False-Positive and False-Negative Results in Urinary Measurement of Albumin or Total Protein**

False-Positive Results	False-Negative Results
Dehydration: increased concentration of protein in urine	Excessive hydration: decreased concentration of protein in urine
Hematuria	Other proteins that do not react with dipstick (eg, monoclonal protein)
Exercise (especially albumin)	
Urinary tract infections	
Extremely alkaline urine (pH > 8)	

- Turbidity is measured with photometer or nephelometer and compared to a standard
- Adequacy of collection ensured by concomitant measurement of urinary creatinine excretion:
  - Normal value <150 mg/d
  - Iodinated contrast material can falsely elevate the protein concentration
- Single voided specimen:
  - Ratio of protein to creatinine concentration in random urine can provide simple estimate of daily protein excretion
  - Best to obtain serial specimens at same time of day given circadian variation of urine protein excretion:
    - Ratio corrects for variations in urine concentration
    - Kidney Disease Outcomes Quality Initiative recommends first morning or random spot urine to monitor proteinuria
  - Good correlation with values obtained by 24-hour specimens once creatinine excretion is known
  - 24-hour specimens or even overnight collections with calculation of the protein-creatinine ratio may lead to better true

estimates of daily urine protein excretion and exclude diurnal variations in protein excretion

**Tests for Albumin Excretion: Methodology**

- Can be quantified in a variety of ways:
  - Radioimmunoassay (gold standard), immunoturbidimetric method, or laser nephelometer are main quantitative methods
  - Semiquantitative dipstick measurements also available for detecting microalbuminuria (albumin >30 µg/min or 30 to 300 mg/d); use limited to screening only; sensitivity, specificity influenced by urine concentration
- Appropriate for early detection of renal disease and cardiovascular risk
- Albumin-creatinine ratios of single voided specimen; timed collections provide better estimate of albumin excretion
- Abnormal values:
  - Microalbuminuria: albumin, 30 to 300 mg/d
  - Albuminuria: albumin >300 mg/d
- In established glomerulopathies, there is no evidence that albuminuria rate is more informative than total proteinuria

**Tests for Light Chains: Methodology**

- Establishes diagnosis of multiple myeloma or other monoclonal gammopathy
- Bence-Jones test: heat:acetic acid precipitation; insensitive and difficult to perform
- Best test is protein electrophoresis, which detects monoclonal peak and immunofixation to identify specific protein

**Spot Versus Timed Urine Collections in Assessment of Proteinuria/Albuminuria**

- See Table 6

**Table 6. Comparisons of Methods for Assessment of Proteinuria**

Random Urine for Albumin-Creatinine Ratio	Morning Urine for Albumin-Creatinine Ratio	Timed Collections for Albumin Excretion
Convenient	Convenient	Inconvenient
Lower creatinine excretion in women: higher values of albumin-creatinine ratio		Not an issue
Lower creatinine excretion in elderly: higher values of albumin-creatinine ratio		Not an issue
Greater creatinine excretion in African Americans: lower values of albumin-creatinine ratio		Not an issue



- Collections of 24-hour or timed urine specimens are associated with high error rate and are inconvenient
- Studies comparing spot urine albumin-creatinine or protein-creatinine ratio with timed specimens have shown correlation coefficients ranging from 0.6 to 0.96
- First morning urine specimen is preferred and shows best correlation with 24-hour value
- Detailed assessments of precision and bias in the accuracy of spot urine specimens versus timed collections is not adequately known

### Interpretation of Proteinuria

- Electrophoretic pattern of urinary proteins (Fig 1):
  - Glomerular proteinuria: albuminuria is hallmark (making up 60% to 90% of total proteinuria)
  - Tubular proteinuria: low-molecular-weight proteins predominate with total urine protein rarely  $>2$  g/d:
    - Impaired tubular reabsorption of low-molecular-weight proteins, or
    - Overproduction of low-molecular-weight proteins, such as light chains in myeloma
  - Selective proteinuria expressed as clearance of IgG over clearance of transferrin is reliable indicator of severity and reversibility of abnormalities of glomerular proteinuria; patients with highly selective proteinuria have milder tubulointerstitial damage, improved prognosis, and better response to therapy:
    - Selectivity index (SI) =  $\frac{\text{urine IgG}}{\text{serum IgG}} \times \frac{\text{serum transferrin}}{\text{urine transferrin}}$
    - $SI \leq 0.10$  highly selective;  $SI \geq 0.11$  and  $\leq 0.20$  moderately selective;  $SI \geq 0.21$  nonselective
- Patterns of proteinuria:
  - Intermittent:
    - Due to hemodynamic alterations in permselectivity
    - Associated with fever, exercise, stress
    - Benign prognosis

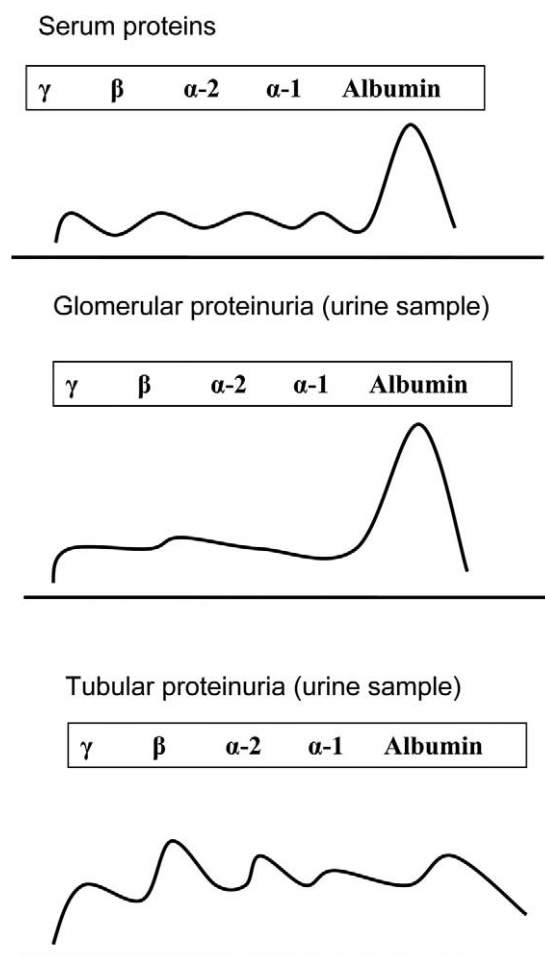


Fig 1. Electrophoretic patterns of serum and urine in patients with abnormal protein excretion.

- Orthostatic:
  - Proteinuria only in erect position with total proteinuria usually  $<1$  g/d
  - Diagnosis with split 24-hour urine specimen: with 16-hour collection while patient upright and 8-hour collection while recumbent
  - Benign condition
- Persistent:
  - Almost invariably sign of structural renal disease and often requires renal biopsy for definitive diagnosis

### ADDITIONAL READING

1. Abuelo JG: Proteinuria: Diagnostic principles and procedures. *Ann Intern Med* 98:186-196, 1983

2. Weber MH: Urinary protein analysis. *J Chromatogr* 429:315-344, 1988

3. Ginsberg JM, Chang BS, Matarese RA, Garella S: Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med* 309:1543-1546, 1983

4. Schwab SJ, Dunn FL, Feinglos MN: Screening for microalbuminuria: A comparison of single sample methods of collection and techniques of albumin analysis. *Diabetes Care* 15:1581-1584, 1992

5. Rodby RA, Rohde RD, Sharon Z, et al: The urine protein to creatinine ratio as a predictor of 24-hour urine protein excretion in type 1 diabetic patients with nephropathy. The Collaborative Study Group. *Am J Kidney Dis* 26:904-909, 1995

6. Zelmanovitz T, Gross JL, Oliveira JR, et al: The receiver operating characteristics curve in the evaluation of a random urine specimen as a screening test for diabetic nephropathy. *Diabetes Care* 20:516-519, 1997